SEAMAP
SOUTHEAST FISHERIES SCIENCE CENTER
ICHTHYOPLANKTON PROTOCOLS

SUBMITTED BY: Joanne Lyczkowski-Shultz

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I. Procedures for processing SEAMAP plankton samples.

A. BONGO SAMPLES

1. Measure plankton displacement volume for all BONGO net samples.

2. When excessively large numbers of fish are present, sort **(in distilled water)** an aliquot of the sample that is sufficient to yield 1,000 fish larvae, juveniles and adults. Clearly note on the data sheet the size of the aliquot sorted for fish larvae.

REMEMBER:
ALL FISH LARVAE MUST BE REMOVED FROM THE ALIQUOT.
AND
MINIMIZE THE TIME SPECIMENS ARE HELD IN WATER DURING PRESORTING AND IDENTIFICATION.

3. In general, the sample should never be split more than 5 times with the Folsom Plankton Splitter.

4. Sort **(in distilled water)** an aliquot of the sample that is sufficient to yield about 200 fish eggs when excessively large numbers of fish eggs are present. Clearly note on the data sheet the size of the aliquot sorted for fish eggs. Bongo samples under the Ich/Sort Eggs/TUNA & Ich/TUNA Protocols may also be aliquoted for eggs but not for larvae.

REMEMBER:
ALL FISH LARVAE MUST BE REMOVED FROM THE ALIQUOT.
AND
MINIMIZE THE TIME SPECIMENS ARE HELD IN WATER DURING PRESORTING AND IDENTIFICATION.

Remember to check the performance of the plankton splitter frequently by allowing the sample splits to settle undisturbed for

twenty minutes on a level surface then compare the amount of sample in each jar to ascertain if the amount of sample in each jar is equal. If the amount of sample in each jar is not equal reposition and/or adjust the splitter, recombine the splits and aliquot the sample again.

5. Place all specimens (fish larvae and fish eggs) into 95% ethanol.

B. **SEAMAP and Spanish** NEUSTON SAMPLES

1. Do not measure displacement volume for neuston net samples.

2. Sort **(in distilled water)** and remove only fish specimens (larvae, juveniles and adults) from the entire sample for those neuston samples denoted by "Ich" in the protocols column of the Prioritized Sorting List. Remove both fish and fish eggs from those neuston samples denoted by "Ich/Sort Eggs" in the protocols column.

3. When excessively large numbers of **fish and/ or eggs** are present, sort **(in distilled water)** an aliquot of the sample that is sufficient to yield 1,000 fish larvae, juveniles and adults. Clearly note on the data sheet the size of the aliquot sorted for fish larvae.

REMEMBER:
ALL FISH LARVAE MUST BE REMOVED FROM THE ALIQUOT.
AND
MINIMIZE THE TIME SPECIMENS ARE HELD IN WATER DURING PRESORTING AND IDENTIFICATION.

4. Place all specimens (fish larvae and eggs) into 95% ethanol.

C. MOCNESS SAMPLES

1. Measure plankton displacement volume for all MOCNESS samples.

2. Sort **(in distilled water)** and remove all fish larvae, juveniles, adults, and fish eggs from MOCNESS samples. **Net 0 samples may be aliquoted. Nets 1-9 samples cannot be aliquoted regardless of the measured plankton displacement volume or the number of eggs and larvae present.**

REMEMBER:

ALL FISH LARVAE MUST BE REMOVED FROM THE ALIQUOT.

AND

MINIMIZE THE TIME SPECIMENS ARE HELD IN WATER DURING PRESORTING AND IDENTIFICATION.

3. Place all specimens (fish larvae <u>and</u> eggs) into <u>95% ethanol</u>.

II. Identification and measurement of ichthyoplankton in SEAMAP samples.

Use distilled water during identification and measurement of larvae. Please minimize the time specimens are held in water during identification and measurement.

A. Identification

1. Please follow the classification and higher level fish names listed in Table 1.

2. Identify specimens of only the following families to the lowest possible taxon (i.e. genus and species): CLUPEIDAE *****, SCIAENIDAE, SERRANIDAE, SCOMBRIDAE, STROMATEIDAE, MUGILIDAE, LUTJANIDAE, and CARANGIDAE.

*** It is now permissible to use the combined name, '*Sardinella/Harengula*', for larvae that belong to one of those two genera but that cannot be reliably distinguished from each other. Write that name on the data sheet and enter that name in the computer.**

3. Identify larvae of all other families to the genus or species level only when such identification can be made easily with little additional time. Otherwise identify to the family level only.

4. Use question marks and general comments to denote or bring attention to an uncertain or "best guess" identification of a specimen. This practice should be used whenever necessary. Make sure that all question marks and comments are entered on the electronic copies of the data sheets. Such useful notes and comments will be appended to the SEAMAP Archiving Center's computerized Comments File, and will be supplied to researchers requesting SEAMAP archived material.

5. Those specimens which cannot be taken to at least the Order level, but which are in identifiable condition, will be classified as 'Unidentified'. It is permissible to use multiple categories of unidentified larvae such as 'Unidentified I' or 'Unidentified II' to denote larvae that are morphologically similar to each other. Head and tail sections that cannot be identified but are in good condition are placed in vials labeled 'Unidentified'.

It is now permissible to use the name 'Disintegrated' to denote damaged or mangled larvae that are unidentifiable. Those larvae in such a poor state that they are impossible to identify are called 'Disintegrated'. Head and tail sections that are too damaged to be identified are also placed in vials labeled 'Disintegrated'.

6. All fish (regardless of size or stage of development) are to be removed from samples, identified, counted, and placed in labeled specimen vials or jars that match the size of the specimens. An exception to this would be when only a few specimens of the taxon would require a larger vial or jar, in that case place all the specimens in the larger jar. Please do not break spines off specimens or force large specimens into small vials. Use larger jars for these specimens (especially the Balistidae).

B. Specimen Counts

1. COUNT the number of specimens of ALL taxa **including** '**Unidentified', 'Disintegrated'**, Clupeiformes, Perciformes, Percoidei etc and record the count and the aliquot of the sample sorted (entire or the fraction) on the data sheet and inside vial label.

2. When samples contain many parts and pieces of larvae of **'Unidentified', 'Disintegrated',** Clupeiformes, Perciformes, Percoidei etc. count only the 'heads'.

Head and tail sections are sometimes present and may possibly be identified. Head sections that can be identified should be included as a number in the total count for whole specimens; if tail sections are recognizable to species, include them in the appropriate species vial, but counting tails is not necessary.

3. Count all fish eggs from bongo net and designated neuston net samples and record what portion (aliquot) of the sample was sorted (entire or the fraction).

4. Place eggs in labeled vials **containing 95% ethanol** and ship them to the SEAMAP Archiving Center in St. Petersburg, Florida as is done with fish specimens.

C. Measurement

REMEMBER:

Specimens to be measured must be chosen randomly from the dish so that the lengths are a random subset of all the larvae in the sample. (Follow instructions that were presented by Dr. Jon Hare at the 2011 Advisory Committee meeting in Szczecin.)

*** = change or revision of previous protocols**

1. Measure up to 10 larvae identified to species for all taxa except the Myctophidae. For myctophid larvae identified to species measure only the smallest and largest specimens.

***** 2. Measure up to **10** specimens of all taxa of snapper larvae including larvae identified as *Lutjanus* or Lutjanidae.

*** 3 a.** Measure body length of **up to 50** specimens identified to genus or family in the **suborder Scombroidei** (i.e. the tunas, mackerels, and related species) **in bongo, neuston and MOCNESS samples from all cruises.**

*** 3 b. Measure** body length of **up to 10** specimens identified to genus or family in the **suborder Scombroidei** (i.e. the tunas, mackerels, and related species) **in Spanish Neuston samples from all cruises.**

***** 4. Except as noted above, measure the smallest and largest specimens only for larvae identified to genus, family, **suborder or order level, e.g. Percoidei, Clupeiformes, Anguilliformes, and Perciformes. Remember these specimens must be counted.**

5. Do not measure specimens of 'Unidentified' or 'Disintegrated' larvae.

6. Use the appropriate measurement of body length, i.e. notochord or standard length, depending on the stage of development of the specimen.

III Data Sheet and Vial or Jar Labeling and Mailing

1. Send all original data sheets and the sorting record sheets to Dr. Joanne Lyczkowski-Shultz at the SEAMAP Laboratory in Pascagoula, Mississippi. **(Keep a copy of all original data sheets until it is confirmed that original data sheets have been received in the U.S.). Also send copies of the computer generated Tuna data sheets to Dr. Lyczkowski-Shultz.** Email Microsoft Excel files containing the data for each SEAMAP cruise to (Joanne.Lyczkowski-Shultz@noaa.gov) when sample identification and data entry checks are completed.

2. Send computer-generated identification data sheets along with the specimens (fish larvae and eggs) to the SEAMAP Archiving Center in St. Petersburg, Florida.

3. Any cephalopods removed from SEAMAP samples are to be placed in individual labeled vials containing 95% ethanol and shipped to Dr. Joanne Lyczkowski-Shultz at the SEAMAP Laboratory in Pascagoula, Mississippi.

3. The TOTAL NUMBER of vials and/or jars in which a taxon has been placed must be recorded in the "vial number" column on the identification data sheet. The label inside a vial or jar containing specimens should specify which vial or jar of the total number of vials or jars it is, for example: 1 of 2, or 2 of 2, etc. This information can be placed on the back of the label.

4. Record whether the larvae or eggs were sorted from the entire sample, denoted by 1; or a 1/2 (or smaller for eggs) aliquot on both the identification data sheets and on inside vial labels.

5. Record the following information on inside vial and/or jar labels: SEAMAP sample number, vessel, cruise number, taxon, number of specimens, aliquot size, and vial number of total vials used for that taxon (to be placed on back of label). Please use preprinted labels provided.

6. Please note which samples have been resorted for quality control and record results of these resorts on the Ichthyoplankton Sorting Record data sheet(s).

IV Shipment of Vials and Jars

1. Packages containing boxes of specimens must be reinforced with shipping tape and plastic or metal banding.

2. Packages containing oversize jars of larger specimens must be packed securely with additional packing material inside the box. These packages should have added reinforcement with shipping tape and banding. Distribute the jars in multiple packages to reduce the weight of individual packages.

3. Packages must be labeled on the outside indicating which package of the total number of packages it represents, for example, 1 of 12 or 5 of 12 etc.

Table 1: This is the classification scheme and higher level names of fishes to be used when identifying larvae from SEFSC/SEAMAP and DISL collections.

Elopiformes
- Elopidae
- Megalopidae

Albuliformes
- Albulidae

Anguilliformes
- Synaphobranchidae
- Moringuidae
- Nettastomatidae
- Congridae
- Ophichthidae
- Anguillidae
- Muraenidae
- Derichthyidae
- Serrivomeridae
- Nemichthyidae
- Chlopsidae
- Heterenchelyidae
- Cyematidae

Saccopharyngiformes
- Saccopharyngidae
- Eurypharyngidae
- Monognathidae

Notacanthiformes
- Notacanthidae

Clupeiformes
- Clupeidae
- Engraulidae

Argentiniformes
- Argentinoidei
  - Argentinidae
  - Microstomatidae
    - Bathylaginae
    - Microstomatinae
  - Opisthoproctidae
- Alepocephaloidei
  - Leptochilichthyidae
  - Alepocephalidae
  - Platytroctidae

Stomiiformes
- Gonostomatidae
- Sternoptychidae
- Phosichthyidae
- Stomioidei
  - Chauliodontidae
  - Stomiidae
  - Astronesthidae
  - Melanostomiidae
  - Malacosteidae
  - Idiacanthidae

Ateleopodiformes
- Ateleopodidae

Aulopiformes

- Aulopoidei
  - Aulopidae
  - Synodontidae
- Chlorophthlamoidei
  - Chlorophthalmidae
  - Ipnopidae
  - Notosudidae
- Alepisauroidei
  - Alepisauridae
  - Evermannellidae
  - Paralepididae
  - Scopelarchidae
- Giganturoidei
  - Bathysauridae
  - Giganturidae

Myctophiformes
- Neoscopelidae
- Myctophidae

Gadiformes
- Bregmacerotidae
- Bathygadidae
- Macrouridae
- Moridae
- Melanonidae
- Phycidae
- Merlucciidae
- Steindachneriidae

Ophidiiformes
- Bythitoidei
  - Aphyonidae
  - Bythitidae
- Ophidioidei
  - Ophidiidae
    - Brotulinae
    - Brotulotaeniinae
    - Ophidiinae
    - Neobythitinae
  - Carapidae

Batrachoidiformes
- Batrachoididae

Lophiiformes
- Lophioidei
  - Lophiidae
- Antennarioidei
  - Antennariidae
- Chaunacoidei
  - Chaunacidae
- Ogcocephaloidei
  - Ogcocephalidae
- Ceratioidei
  - Caulophrynidae
  - Centrophrynidae
  - Ceratiidae
  - Diceratiidae
  - Gigantactinidae
  - Himantolophidae
  - Linophrynidae
  - Melanocetidae
  - Neoceratiidae
  - Oneirodidae
  - Thaumatichthyidae

Atheriniformes
- Atherinidae
- Atherinopsidae

Mugiliformes
- Mugilidae

Cyprinodontiformes

Beloniformes
- Scomberesocidae
- Belonidae
- Hemiramphidae
- Exocoetidae

Lampridiformes
- Lamprididae
- Lophotidae
- Radiicephalidae
- Trachipteridae
- Regalecidae
- Stylephoridae

Beryciformes
- Anomalopidae
- Anoplogasteridae

- Berycidae
- Diretmidae
- Holocentridae
  - Holocentrinae
  - Myripristinae
- Trachichthyidae

Stephanoberyciformes
- Stephanoberycidae
- Melamphaidae
- Gibberichthyidae
- Rondeletiidae
- Barbourisidae
- Cetomimidae
- Mirapinnidae
- Megalomycteridae

Polymixiiformes
- Polymixiidae

Zeiformes
- Parazenidae
- Zeidae
- Grammicolepidae
- Oreosomatidae
- Zeniontidae

Syngnathiformes
- Aulostomidae
- Centriscidae
- Fistulariidae
- Syngnathidae
  - Hippocampinae
  - Syngnathinae

Scorpaeniformes
- Scorpaenidae
- Triglidae
- Peristediidae
- Psychrolutidae

Perciformes
Percoidei
- Centropomidae
- Acropomatidae
- Polyprionidae
- Howellidae
- Serranidae
  - Anthiinae
  - Epinephelinae
    - Grammistini
    - Liopropomatini
  - Serraninae
- Symphysanodontidae
- Grammatidae
- Priacanthidae
- Caproidae
- Apogonidae
- Epigonidae
- Malacanthidae
- Dactylopteridae
- Scombropidae
- Pomatomidae
- Rachycentridae
- Echeneidae
- Carangidae
- Coryphaenidae
- Bramidae
- Caristiidae
- Emmelichthyidae
- Lutjanidae
  - Apsilinae
  - Etelinae
  - Lutjaninae
- Lobotidae
- Gerreidae
- Haemulidae
- Inermiidae
- Sparidae
- Sciaenidae
- Polynemidae
- Mullidae
- Pempheridae
- Bathyclupeidae
- Kyphosidae
- Ephippidae
- Chaetodontidae
- Pomacanthidae
- Pomacentridae
- Cichlidae
- Cirrhitidae
- Opistognathidae

Labroidei
- Labridae
- Scaridae

Zoarcoidei
- Zoarcidae

Trachinoidei
- Chiasmodontidae
- Ammodytidae
- Uranoscopidae
- Percophidae

Blennioidei
- Tripterygiidae
- Chaenopsidae
- Dactyloscopidae
- Labrisomidae
- Blenniidae

Gobiesocoidei
- Gobiesocidae

Callionymoidei
- Callionymidae
- Draconettidae

Gobioidei
- Eleotridae
- Gobiidae
- Microdesmidae
- Ptereleotridae

Acanthuroidei
- Luvaridae
- Acanthuridae

Sphyraenoidei
- Sphyraenidae

Scombroidei
- Scombrolabracidae
- Gempylidae
- Trichiuridae
- Scombridae

Xiphioidei
- Istiophoridae
- Xiphiidae

Stromateoidei
- Centrolophidae
- Nomeidae
- Ariommidae
- Tetragonuridae
- Stromateidae

Pleuronectiformes
- Scophthalmidae
- Paralichthyidae
- Bothidae
- Pleuronectidae
- Poecilopsettidae
- Achiridae
- Cynoglossidae

Tetraodontiformes
- Triacanthodidae
- Balistidae
- Monacanthidae
- Ostraciidae
- Tetraodontidae
- Diodontidae
- Molidae